

In vivo x-ray diffraction of indirect flight muscle from *Drosophila melanogaster*

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Introduction

Small-angle x-ray diffraction from isolated muscle preparations is commonly used to obtain time-resolved structural information concerning the molecular events occurring during muscle contraction. While most x-ray studies to date have looked at vertebrate muscle from frogs (and less commonly, rabbits), indirect flight muscle from large insects such as *Lethocerus* have been shown [1, 2] to give very high-quality x-ray patterns because of their higher degree of crystalline order. We have extended this technique to the thoracic flight muscles of living fruit flies at rest and during tethered flight. Fruit flies are small organisms, allowing truly *in vivo* measurements. They are the best understood genetically of all eukaryotic organisms allowing one to undertake structure and function studies of indirect flight muscles from mutant flies. This new experimental system facilitates investigation of the relation between molecular structure and muscle function in living organisms.

Methods and Materials

The experiments were carried out at the Bio-CAT undulator-based beamline at the Advanced Photon Source at Argonne National Laboratory. Adult flies (2–3 day old females) were held at right angles to the x-ray beam while allowing unimpeded movement of the wings and legs (Fig. 1). The collimated beam intercepted all 12 dorsal-longitudinal muscle (DLM) fibers located in two muscle sets on each side of the median plane of the thorax. Time-resolved experiments employed a rotating shutter to isolate two phases of the wing beat cycle.

Results

Figure 2 shows a resting pattern from the DLM of a wild-type fly. This pattern shows many similarities to those obtained from glycerinated fibers isolated from *Lethocerus* [1, 2]. Both patterns show better lattice ordering than those from vertebrate muscle, with numerous diffraction peaks on the equator, and layer lines that index as various orders of a 232 nm repeat [1]. All of these diffraction features are candidates for time-resolved experiments that aim to elucidate various aspects of acto-myosin interaction.

Precise measurements at 1 millisecond time resolution indicate that the myofilament lattice spacing does not change significantly (within 1 Å) during oscillatory contraction. This result is consistent with the notion that a net radial force maintains the thick filaments at an 'equilibrium' interfilament spacing of ~56 nm throughout the contractile cycle,

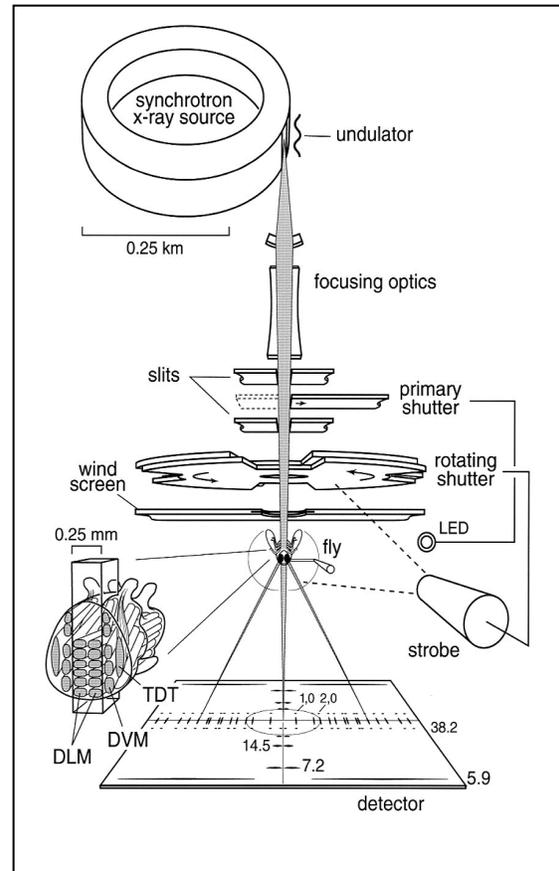


Figure 1: Fly wing and leg movement is unimpeded in the apparatus setup.

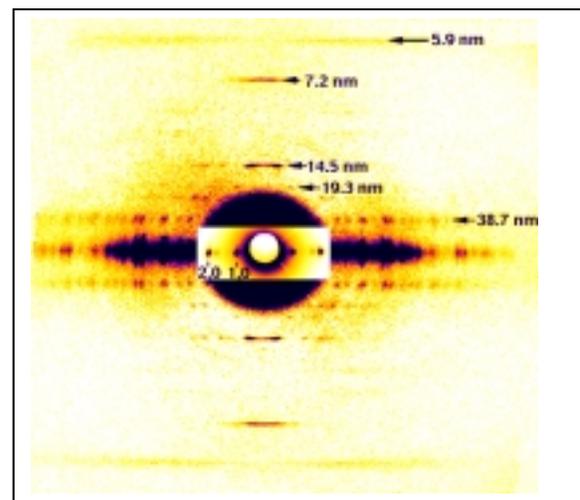


Figure 2: Resting pattern from the DLM of a wild-type fly.

which is probably the optimum spacing for acto-myosin interaction. Transgenic flies with amino acid substitutions in the conserved phosphorylation sites of the myosin regulatory light chain (RLC) exhibit structural abnormalities that can explain their flight impairment. The I20/I10 equatorial intensity ratio of the mutant fly is 35% less than that of wild type, supporting the hypothesis that myosin heads that lack phosphorylated RLCs remain close to the thick filament backbone.

More recently we have developed a demembrated (“skinned”) preparation, which allows a large span of other potential experiments [3].

Discussion

In summary, we have used *Drosophila* to obtain detailed, two-dimensional x-ray diffraction patterns of working muscle in a living organism. We demonstrated a new application of coupling the molecular genetic toolkit of *Drosophila* to high-resolution biophysical measurements. The *in vivo* technique complements experiments on excised, skinned preparations, and advances *Drosophila* as a model system for studying integrative biology [4]. The demonstrated ability to carry out x-ray studies in transgenic flies with defined alterations of muscle proteins will increase our understanding of how they function *in vivo*. (A fuller report of the work presented here appeared in the May 2000 issue of *Biophysical Journal*.)

Acknowledgments

DWM acknowledges support from the Office of Naval Research and the National Science Foundation. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-ENG-38. BioCAT is a U.S. National Institutes of Health-supported Research Center (RR08630).

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